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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: ROBERT E. JOHNSTON, ET AL. ART UNIT:
SERIAL NO.: 10/683,781 EXAMINER:
FILING DATE: OCTOBER 10, 2003
FOR: ALPHAVIRUS RNA REPLICON SYSTEMS

DECLARATION JONATHAN F. SMITH, PH.D., 37 C.F.R. §1.608(b)

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SIR:

I, Jonathan F. Smith, Ph.D., do hereby declare and state:

1. I am a virologist specializing in vaccine development and I earned my Ph.D. in Microbiology and Virology from the University of Texas at Austin. I conducted postdoctoral studies at the Institute for Genetics at the University of Cologne in Germany and then joined the faculty of the University of Maryland School of Medicine, Department of Microbiology as an Assistant Professor. I was the Chief of the Department of Viral Biology in the Virology Division of the United States Army Medical Research Institute of Infectious Diseases (USAMRIID), where I worked as a scientist for 17 years. Currently, I am the Chief Scientific Officer at AlphaVax, Inc. While at USAMRIID I contributed, through my investigative work, to inventing the Alphavirus RNA Replicon Systems of U.S. Patent Application Serial No. 10/683,781. In preparing this declaration I reviewed the following documents:

Exhibit A	Copy of USAMRIID Notebook #3656, VEE Replicon (Dr. Pushko's entries)
Exhibit B	Data Summary I, pp. 1-4, summary of #3656 experiments, 9/11/94 to 12/26/94

Exhibit E	Letter from Dr. Nancy Davis to Dr. Jonathan Smith, July 7, 1992
Exhibit F	Letter from Dr. Nancy Davis to Dr. Jonathan Smith, May 24, 1993
Exhibit G	ILIR Research Proposal for Funding the Alphavirus RNA Replicon System Project
Exhibit H	Evaluation for Associate (Dr. Pushko) by Research Advisor (Dr. Smith)

2. My involvement (concept development and laboratory research) in developing the Alphavirus RNA Replicon System invention of U.S. Patent Application Serial No. 10/683,781 ("the '781 application") is discussed herein below. I understand that the time period spanning from November 30, 1994 through May 23, 1995 is being referred to as the Activity Time Period, however, my contributions occurred both prior to and during the Activity Time Period.

3. The Alphavirus RNA Replicon System was conceived well prior to November 30, 1994, and I contributed to formulating the original ideas about how to utilize the system as a vaccine. The fundamental and detailed concept of the project was developed during discussion with collaborating scientists Nancy Davis ("Davis") and Robert E. Johnston ("Johnston") at the University of North Carolina at Chapel Hill (UNC-CH) and Michael Parker ("Parker") and George V. Ludwig ("Ludwig") of the Virology Division at USAMRIID. I understand that Davis, Johnston, Parker, and Ludwig are designated co-inventors of the subject matter of the '781 application, and in particular, Claim 116, described below.

4. Correspondence sent to me from Davis reflects the discussions we and others, including at least Johnston and Parker, had prior to November 30, 1994 regarding the development of the Replicon System. These letters further document the exchange of attenuated Venezuelan Equine Encephalitis virus ("VEE") genomic sequences that were utilized to construct the VEE replicons and replicon helpers, such as those disclosed in the '781 application. (Exhibit E, letter of July 7, 1992 and Exhibit F, letter of May 23, 1993).

5. Following numerous discussions with Davis and Johnston, Parker and I wrote a short

in-house research proposal, referred to as an ILIR proposal, to obtain preliminary funding support for the alphavirus replicon studies to be conducted at USAMRIID. The alphavirus that is described in the proposal is the VEE virus. (Exhibit G, pages 2 - 5, ILIR Proposal).

6. The ILIR proposal was first reviewed by the Virology Division Chief, Jeffrey Chulay and then submitted to the Office of Research Plans and Programs at USAMRIID with a cover memo from him. The cover memo prepared by Jeffrey Chulay is dated "4 August, 1993" and the date stamp of "09 Aug 1993" in the upper right corner of the memo reflects the date the ILIR proposal and memo was received for review by the Office of Research Plans and Programs (Exhibit G, front page).

7. The ILIR proposal describes the invention that is the subject of the '781 application and specifically describes the exemplary alphavirus replicon and helper sequences that were developed from VEE. The proposal documents that we had understood all essential elements of the invention and how we (Parker, Davis, Johnston and myself) planned to create the desired replication defective alphavirus replicon particles that would be capable of producing an immune response to an immunogen of choice. We understood this to require the development of replicon sequences, the desired heterologous RNA sequence(s) (encoding an immunogen), and replicon helper sequences expressing the necessary structural proteins for packaging the replicon into infectious particles.

8. The ILIR proposal describes the replication defective VEE particles as containing a replicating RNA sequence, referred to as the replicon (Exhibit G, page 2, last ¶), that would be packaged into infectious particles, akin to the packaging of the wild-type virus genome. The replicon RNA sequence contains intact viral replication and transcription functions. When transfected into cells the replicon encoded nonstructural protein gene(s) are translated into protein(s), which then function to direct replication of replicon RNA through a complementary

strand intermediate. (Exhibit G, page 2, ¶3). The replicon also contains a coding sequence(s) for the immunogen(s) of choice that is expressed from the complementary strand intermediate and subsequently translated into protein by the infected cell (Exhibit G, pages 3-4).

9. The VEE structural proteins, which form the "coat" or particle around the RNA replicon, are provided by an additional RNA sequence(s), referred to as the helper sequence (Exhibit G, page 2, ¶ bridging page 3). In order to prevent the formation of fully virulent replication competent particles, the helper sequences included attenuating mutations and/or deletions in at least one structural protein gene.

10. The ILIR proposal describes incorporating attenuating mutations in VEE structural protein genes in the helper sequences used to produce infectious replication defective VEE particles from the engineered VEE replicons. (Exhibit G, page 2, e.g., pV3507 disclosed in the '781 application).

11. Thus, in addition to designing replicon and helper RNA protocols necessary for RNA production, transfection and expression methods were also developed. These included RNA transcription reactions, electroporation and other transfection protocols, calculations of transfection efficiency, detection of RNA translation (e.g., VEE structural protein gene products (i.e., a particle protein)) and the immunogenic protein(s) of interest. Various protocols, such as transfection, were optimized in my laboratory and numerous assays developed for assessing infectivity of the replicon particle, expression of the immunogen in vitro, and assessment of replication incompetence. These included a combination of plaque assay (i.e., passage on permissive cells in culture) and immunofluorescence assays (IFA) using antibodies to the immunogen such as Lassa fever nucleoprotein (LSN) and a VEE encoded structural protein. Many of these tasks were undertaken at both USAMRIID and UNC-CH. In addition, the replicon particles were to be administered to rodents at USAMRIID (Exhibit G, page 4) to demonstrate

the immune response in vivo.

12. The antigens encoded by the heterologous RNAs, including LSN, were antigens known to stimulate an immune response if the encoding gene was expressed and we observed high expression levels of LSN protein from the replicon in vitro. Antigen expression was expected to be independent of the helper sequence used to generate the replicon particles because the helper is not present in the infectious particles.

13. We intended the invention to be used as a vaccine in a variety of mammals, including humans, which therefore required administering the replicon particles to a subject. Thus, in vivo rodent studies were part of our original conception, as is documented in the ILIR (Exhibit G, page 4). The ILIR further described that in vivo studies would assess any immune response to the VEE particle proteins and the ability to boost (i.e. subsequently administer VEE replicon particles and increase the immune response to the same or different antigen (Id.).

14. I understand that pending Claim 116 of the '781 application reads as follows:

Claim 116. A method of producing an immune response in a subject, comprising:

administering to the subject an effective amount of a composition comprising a population of infectious, defective alphavirus particles in a pharmaceutically acceptable carrier,

wherein each particle comprises one or more heterologous RNA sequence(s) encoding an immunogen; and further wherein the population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture.

15. The ILIR proposal describes the invention set forth in Claim 116. The "infectious, defective alphavirus particles" comprising "one or more heterologous RNA sequence(s) encoding an immunogen" is equivalent to the VEE replicon particles encoding the LSN antigen that were produced prior to November 30, 1994 in my lab. The production of such particles inherently requires the successful development of a helper sequence(s) that can produce the requisite

structural packaging proteins.

16. In addition, the phrase "wherein the population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture" generically describes the approach outlined and used to assess the VEE particles produced in my lab at USAMRIID. For example, the supernatant of VEE replicon particle-infected cells when incubated with Baby Hamster Kidney (BHK) cells resulted in fewer than 100 plaque forming units per ml of supernatant (pfu/ml), which is the lower limit of detection of the plaque assay. Thus, plaque assays were used to verify that the packaged replicons that were "replication defective" or, in other words, incapable of initiating a productive infection.

17. In my opinion, the required elements for the method of Claim 116 were identified by myself and co-workers prior to November 30, 1994 when we demonstrated that a VEE replicon particle encoding the LSN antigen was packaged into particles that (1) infected permissive cells, (2) expressed high levels of the LSN antigen, and (3) failed to produce measurable plaques by plaque assay (Exhibit B, p. 3, top row, October 13, 1994, LSN replicon particles, produced using the P1-5 helper, infected permissive cells, expressed high levels of LSN antigen shown by IFA (Exhibit A, p. A091, IFA II) and were replication defective as determined by passage on permissive cells (i.e., <100 pfu by plaque assay; Exhibit A A096, samples 10-13) and similarly for LSN replicon particles produced with the P5-1 helper, November 17-25, 1994 (Exhibit A, "zero" plaques for specimen # 33, p. A122 and p. A123, row 5 and Exhibit B, p. 1, last row). The method of Claim 116 was first practiced in the first animal study designed and conducted by Ludwig at the time he measured the immunogenic response to the LSN antigen in mice immunized with the LSN replicon, which was expressed by the infectious replication defective replicon particles.

18. I was responsible for arranging a one year research associate position in my

laboratory for Peter Pushko ("Pushko"), a co-inventor of the subject matter claimed in the '781 application who was brought to USAMRIID specifically to develop the reagents and protocols mentions above. In addition, I supported the renewal of Pushko's position in the lab, as is evidenced in an evaluation I submitted approximately one year after he began collaborating on the alphavirus replicon project (Exhibit H, Evaluation of Associate by Research Advisor). I understand that Pushko is designated as a co-inventor of the subject matter of the '781 application, and in particular, Claim 116.

19. Pushko's laboratory work at USAMRIID on the VEE replicon, as is shown in notebook #3656 (Exhibit A), was conducted by Pushko upon his arrival at USAMRIID. Notebook #3656 was originally assigned to Parker (see, Exhibit A, notebook cover page) but given to Pushko to record his work on the Alphavirus RNA Replicon System.

20. During the Activity Time Period of November 30, 1994 through May 23, 1995, I was actively involved in the Alphavirus RNA Replicon System project and served in a supervisory and consulting capacity for Pushko. Thus, I was involved in reviewing data and experimental results on a regular basis (i.e., no less than weekly) during the Activity Time Period.

21. Of the co-inventors at USAMRIID, I was primarily responsible for the ongoing collaborative communications with co-inventors Davis and Johnston of UNC-CH. We exchanged correspondence and spoke via telephone throughout the Activity Time Period on at least a weekly basis. Similarly, I was the person at USAMRIID responsible for exchanging reagents with Davis and Johnston that pertained to the development of the Alphavirus RNA Replicon System.

I, Jonathan F. Smith, Ph.D., declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that any such willful false statements may jeopardize the validity of U.S. Patent Application Serial No. 10/683,781 or any patent issuing therefrom.

9-1-04
Date

Jonathan Smith
Jonathan F. Smith, Ph.D.